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Suppression of *Salvinia molesta* Mitchell in Texas and Louisiana by *Cyrtobagous salviniae* Calder and Sands

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Abstract

Release and evaluation studies of the Brazil population of *Cyrtobagous salviniae* on *Salvinia molesta* were conducted originally at 18 sites in Texas and Louisiana from 1999 through 2005. However, overall project results could only be continually evaluated at two release and two control sites because the remainder were eventually destroyed or corrupted by floods, droughts, or herbicides. Mean fresh weight biomass of *S. molesta* ranged from 15.5 kg FW m^{-2} during the summer to as low as 2.1 kg FW m^{-2} during the winter prior to the release of *C. salviniae*. Insect populations established within a year of release and initially spread slowly. Damage to *S. molesta* increased with increasing *C. salviniae* detections while *S. molesta* biomass and surface coverage declined at both release sites by more than 99% while remaining unchanged at the control sites. Water in release sites registered higher levels of dissolved oxygen, higher temperatures, and higher pH than water in control sites. This study provides another example of the effectiveness of *C. salviniae* against *S. molesta* even in more temperate climates.
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Keywords: Giant salvinia; *Salvinia molesta*; *Cyrtobagous salviniae*; Classical biological control

1. Introduction

Giant salvinia, *Salvinia molesta* Mitchell, is a cosmopolitan pest of temperate to tropical freshwater ecosystems. It has spread through the agency of man, from its geographic origin in South-Eastern Brazil, to Africa, Asia, Australia, and Central and North America (Room, 1990). This plant is a sterile polyploid so reproduction is entirely vegetative (Loyal and Grewal, 1966). *S. molesta* forms chains of ramets linked by a horizontal rhizome which fragments with age and damage to form new plants, thereby facilitating spread (Room, 1983). Its unregulated growth and floating habit result in rapid and often complete coverage of stagnant water bodies, with inevitable spread throughout contiguous drainages via currents or flooding events. The thick mats formed by this plant disrupt or prevent commercial and recreational activities such as boating and fishing (Thomas and Room, 1986). In addition, *S. molesta* mats reduce open water which inhibits the direct diffusion of oxygen into the water column (Ruttner, 1952). Submerged plants are shaded out, thereby reducing the production of photosynthetic oxygen which, coupled with the consumption of dissolved oxygen by decaying *S. molesta*, further reduces dissolved oxygen while increasing carbon dioxide and hydrogen sulphide concentrations, a scenario usually detrimental to fisheries (Mitchell, 1972; Thomas and Room, 1986). Worldwide, *S. molesta* mats have provided ideal habitat for *Mansonia* sp. and other mosquitoes which vector human diseases like encephalitis, dengue fever, and malaria (Creagh, 1991). Two species of *Mansonia* in the US, *M. dyari* (Belkin) and *M. titillans* (Walkter) (Diptera: Culicidae), have been implicated in the transmission of St. Louis encephalitis and Venezuelan equine encephalitis, respectively (Lounibos et al., 1990).

In general, mechanical and chemical controls in large drainages have proven economically impractical because they must be applied repeatedly and indefinitely (Thomas and Room, 1986). Surviving plants or fragments often persist undetected in and among emergent vegetation and provide a nucleus for rapid recolonization. Projects utilizing classical biological control were initiated as a result in order to

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produce a practical and sustainable solution to this weed problem.

The first attempts at classical biological control targeted *S. molesta* in Africa and Fiji but were foiled by a misidentification of the target weed as *S. auriculata* Aubl., which led to the introduction of ineffective natural enemies like *Cyrtobagous singularis* L. (Coleoptera: Curculionidae) collected from *S. auriculata* (Thomas and Room, 1986). Mitchell (1972) distinguished *S. molesta* from *S. auriculata* and Forno and Harley (1979) eventually discovered the native range of *S. molesta* in South Eastern Brazil. The plant was attacked by a suite of herbivores similar to those on *S. auriculata*, including what was thought to be *C. singularis*. However, this previously ineffective weevil was selected for further studies in the hope it would be better adapted to *S. molesta*. Calder and Sands (1985) reexamined this weevil after its introduction and strong performance on *S. molesta* in Australia and determined that it was a new species which they named *C. salviniae*. Since then it has been employed successfully in at least 15 countries to reduce the dominance of *S. molesta* in invaded freshwater ecosystems (Julien and Griffiths, 1998).

S. molesta was first detected in North America at a small pond in South Carolina in 1995 where it was eradicated with herbicides (Johnson, 1995). Extensive infestations were later discovered in large drainages in Texas during 1999 (Jacono, 1999). It occurs in at least 12 states and new infestations are found regularly, one of the latest in central Florida near Ocala (R. Kipker, pers. comm.). We dealt with the most serious infestations of *S. molesta* in Texas and Louisiana during June 1999 to May 2005 with the objective of introducing and evaluating the Brazilian population of *C. salviniae* as a biological control solution to the problem in the these areas.

2. Materials and methods

2.1. Origin of C. salviniae

Cyrtobagous salviniae adults from the Brazil population were field collected in Australia and South Africa and consigned to the USDA-ARS Invasive Plant Research Laboratory containment facility at Gainesville, Florida where they were reared awaiting resolution of pathogenic, taxonomic, and regulatory issues. South African *C. salviniae* originated from field collections in Namibia which were the progeny of *C. salviniae* that were collected originally in Brazil and released in Australia (Cilliers, 1991). Adults from each source were kept separate and sampled frequently for the presence of pathogens. An unknown *Helicosporidium* sp. (a parasitic alga) was found in adults from the South African source material which forced us to destroy that colony (White et al., 2007). No diseases were found in the Australian colony which was retained as stock for release.

2.2. Field sites

Field study sites were randomly assigned as release sites, where insects were released, or control sites with no insect releases, so that a site constituted the experimental unit. Sites were a minimum of 10 km apart to prevent dispersal of insects into control sites and consisted primarily of ponds but also included some lake, river, and canal locations that were heavily infested with *S. molesta*. We started with a total of 18 sites, 10 of which were designated as release sites (Table 1). The dynamic nature of water combined with a floating and, therefore, mobile plant predisposed the field sites to disruption and perturbations and consequently many of them were compromised or destroyed over the course of this

Table 1
Locations, descriptions, outcomes, and longevity of control (C) and release (R) sites used in evaluation studies with *Cyrtobagous salviniae* (CS) in Texas and Louisiana from 1999 to 2005

Site name	GPS coordinates		Site type	# CS released	Site description and outcome	Site longevity (months)
Crockett	31°12′13.14″N	95°27′09.54″W	C	0	Pond, uninterrupted use	64
TC Outfitters	29°56′23.82″N	94°43′15.54″W	C	0	Pond, uninterrupted use	62
Twin Oaks	31°25′00.31″N	93°40′04.40″W	C	0	Canal, sprayed by state	19
Lake Texana	28°57′24.42″N	96°32′49.80″W	C	0	Lake, vandalized repeatedly, abandoned	11
Shotgun Cove	29°01′52.74″N	96°33′12.24″W	C	0	River, vandalized repeatedly, abandoned	10
Breakleg	30°01′27.08″N	93°47′38.95″W	C	0	Marsh, destroyed by saltwater intrusion	6
Big Gator	29°02′28.74″N	96°34′7.08″ W	C	0	River, destroyed by flooding	5
Duckwish	29°01′18.12″N	96°34′18.12″W	C	0	Oxbow, dried up	5
Cypress Bend	31°26′05.34″N	93°40′46.92″W	R	992	Pond, uninterrupted use	56
Nelson Pond	29°52′05.16″N	94°58′33.61″W	R	740	Pond, uninterrupted use	56
Horseshoe	29°01′47.10″N	96°33′36.24″W	R	658	Oxbow, repeated flooding, sprayed by river authority	50
Swinney Marsh	29°56′06.24″N	94°44′48.46″W	R	694	Oxbow, property sold, new owner closed site	38
Salter Creek	31°27.0413″N	93°38′26.63″W	R	95	Bayou, dried up	16
Carrice Creek	31°27′48.07″N	93°46′32.03″W	R	659	Bayou, repeatedly flooded and dried	14
Briggs Pond	30°02′55.98″N	93°47′39.74″W	R	193	Pond, destroyed by saltwater intrusion	12
Harris Pond	29°55′57.73″N	94°44′37.32″W	R	688	Pond, sprayed by owner	10
Cattail Island	29°01.16.26″N	96°34′25.20″W	R	0	Oxbow, dried up	5
River Island	29°01′56.58″N	96°34′03.30″W	R	0	River, destroyed by flooding	5

study by flooding, drought, saltwater intrusion or herbicide applications. Despite these challenges, we were committed to evaluating this organism under actual field conditions. By the time the study was completed in 2005, there were only two release and two control sites that had been completely unaffected and thus could be compared directly over most of the entire period.

2.3. Study design

We evaluated the uniformity of the *S. molesta* mats in full sun taking four 0.1 m^2 samples of *S. molesta* fresh weight biomass at four locations within a site. All live plants were removed from the sample frame, lightly compressed to remove excess water and weighed to estimate fresh weight biomass. Distances between locations was maximized and sampling was done at four sites over three dates. The results indicated that a mature *S. molesta* mat within a full-sun site tended to be relatively uniform in terms of biomass and growth forms (data not shown), so sampling without apparent bias over time at one location was sufficient to characterize each site. We created a reference location at each field site with a floating square of a pvc pipe (7.6 cm diameter) which enclosed 1 m^2 of the resident *S. molesta* population. The reference frame was anchored with a nylon rope tied to a cinderblock and most were placed close to the bank for access by wading. This provided a reference point for each sampling date and fixed a population of the plant in place for conducting *C. salviniae* releases.

Sampling was conducted every 4–6 weeks during the spring, summer, and fall during a 5-year period from September 1999 to June 2005. We generally did not sample during December, January, and February when biological activity was minimal because of low temperatures. Water conditions were recorded just below the surface and at 1 m depth during each sampling event. We used a variety of calibrated automated hand-held meters to measure temperature, dissolved oxygen, and pH. Four samples of *S. molesta* were collected using 0.1 m^2 frames of pvc pipes as before which were placed without apparent bias on the mat in the vicinity of the reference frame to estimate fresh weight biomass. We estimated the percentage, to the nearest 10%, of the water's surface on the water body covered with *S. molesta* by combining the visual estimates of least two observers. Brown coloration of mats has been associated with insect-damaged and weakened plants (Room et al., 1981), so a visual estimate was also made of the percentage of the mat that appeared green vs. brown, estimated to the nearest 25% within the ranges of 0, 1–24, 25–49, 50–74, 75–99, and 100% green. One hundred terminal buds were collected at each sample date and examined visually *in situ* and rated as damaged or undamaged.

Assessing bud damage and linking it to impacts on *S. molesta* was problematic because of the presence of *Samea multiplicalis* (Guenée) (Lepidoptera: Pyralidae) and *Synclita obliteralis* (Walker) (Lepidoptera: Pyralidae), whose larva feed indiscriminately in the above-water portions of the plant and damaged buds at all sites. We could not consistently distinguish the source of bud damage from among the three herbivores so we elected to tally all damaged buds.

C. salviniae adults found during this examination were recorded and categorized as light or dark brown. Teneral adults remain light brown for a few days before becoming fully sclerotized and turning darker. The presence of these newly emerged adults is indicative of a reproducing population. We also searched periodically for adults on other vegetation in the vicinity of the reference frame by examining adjacent floating, emergent, and terrestrial species for variable periods ranging from 10 to 20 min at each site at most sample dates.

A transect was established perpendicular to the bank through each reference frame to quantify the local spread of released *C. salviniae*. Two 0.1 m^2 samples were collected at 1, 5, and 10 m distances from the side of the reference frame that was opposite the bank of the water body. The same data as above were captured except 50 *S. molesta* buds were examined per sample instead of 100. Plant samples were then replaced in their original locations.

2.4. C. salviniae releases

A total of 5069 adults were released over the period of this study at a variety of locations (Table 1). Only 220 adults were released at Cypress Bend in October 2001. An additional 772 adults were released in 2002 after overwintering from the first release was confirmed. The same number of insects was released initially at Nelson Pond within 24 h of the release at Cypress Bend. No further releases were done until overwintering was evaluated and then another 520 adults were released during 2002.

At the completion of the study, 100 adults were released into each of the two control sites (TC Outfitters, Crockett) starting in April 2004 with another 75 adults released per site in June 2004. This was done as part of an agreement with the landowners who permitted us to use their properties as experimental controls in exchange for us releasing *C. salviniae* at the end of the study.

2.5. Data analysis

Analysis of variance was used to compare differences among the longest running release and control sites with the greatest degree of sampling overlap, namely Nelson Pond and Cypress Bend (release) with Crockett and TC Outfitters (control) (SAS Institute, 1999). Other sites could not be included because of their variable lifespans before they were corrupted or destroyed. The loss of so many sites over the course of the study was regrettable because it left only 2 replications, instead of the original 10 and 8 replications for release and control sites, respectively. Despite this relative loss of statistical power, the magnitude of the differences and the consistency of variances between treatments convinced us to pool the data for analyses using two sample *t*-tests instead of considering sites individually (SAS Institute, 1999). We further partitioned the data into pre- (1999–2001)

and post-release (2002–2005) intervals before comparing results from site types. Values were transformed using appropriate methods when distributions were non-normal or when variances were heterogenous and back transformed for presentation.

3. Results

3.1. Insect recovery and local dispersion

Conclusive evidence of overwintering was first found on March 25, 2002 at the Cypress Bend and Lake Texana sites where adults had been released the previous October (Tipping and Center, 2003). The first teneral adult was collected 219 days post-release at the Nelson Pond site indicating the presence of a breeding population. Since then adults were found every spring during 2002–2005 at the two surviving sites. Insects were also recovered from four other sites before those sites were lost but, because of timing, we could not confirm that overwintering had occurred.

Local spread of *C. salviniae* was initially very slow; most adults were recovered only directly adjacent to the reference frame within months of their release. For example, the first adults were found at a mean maximum distance of 1 m outside of the reference frame 253 days after release at Cypress Bend. This low rate of dispersal was probably influenced by the cooler temperatures of the winter and spring which accounted for most of this interval (October 2001–June 2002). The rate of spread increased during the warmer months after June and adults were detected 5 m away by 277 days post-release, then 10 m away after 359 days.

3.2. Impact of C. salviniae on S. molesta

The mean (±S.E.) fresh weight biomass of *S. molesta* varied over time, reaching as high as 15.5 (±2.8) kg FW m^{-2} during the warmer periods and declining to as low as 2.1 (±0.1) kg FW m^{-2} during the winter prior to the release of insects (Fig. 1). Mean fresh weight biomass at two control sites (Crockett, TC Outfitters) and two release sites (Nelson Pond, Cypress Bend) was not different during the pre-release interval with a mean (±S.E.) of 10.2 (±1.3) and 12.8 (±1.4) kg FW m^{-2} for control and release sites, respectively ($t = 1.31$, d.f. = 46 and $P = 0.197$) (Table 2). Fresh weight biomass was less at release sites during the post-release interval ($t = 7.79$, d.f. = 84 and $P < 0.0001$) with a mean of 8.8 (±0.6) kg FW m^{-2} at control sites and 2.6 (±0.4) kg FW m^{-2} at release sites (Table 2). Although there was no difference in mean percent bud damage between site types before the introduction of the biological control agent ($t = 0.15$, d.f. = 45 and $P = 0.88$), a greater percentage of buds were damaged in the release site during the post-release interval ($t = 2.39$, d.f. = 81 and $P = 0.01$) (Table 2).

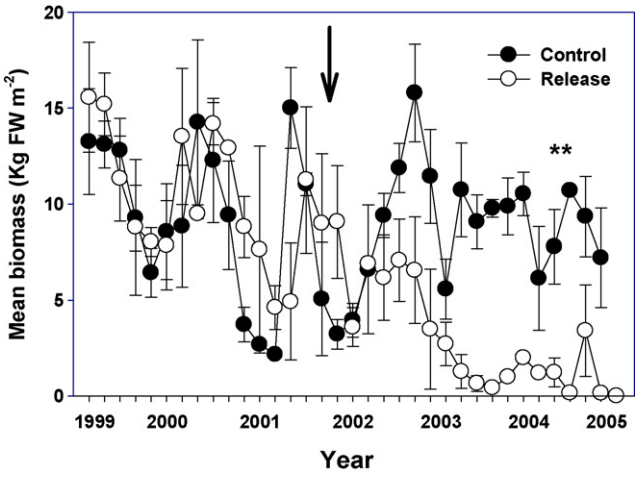

Fig. 1. Mean (±S.E.) fresh weight biomass of *S. molesta* at control and release sites during 1999–2005. A total of 13 sites with a minimum longevity of 10 months are represented for various intervals throughout the course of the study. Two control sites (Crockett and TC outfitters) and two release sites (Cypress Bend and Nelson Pond) are included throughout the entire sampling period. Arrow represents date of first release of *C. salviniae* at release sites and double asterisk represents first release at control sites.

There was no also difference in the percentage of the water surface covered with *S. molesta* between site types during the pre-release interval ($t = 0.05$, d.f. = 40 and $P = 0.96$) while surface coverage was lower at release sites during the post-release interval ($t = 7.30$, d.f. = 84 and $P < 0.0001$) (Table 2).

Table 2
Salvinia and environmental variables at release and control sites during pre- (1999–2001) and post- (2002–2005) release of *C. salviniae*

Variable	Pre-release period			Post-release period		
	Control	Release	*t*	Control	Release	*t*
Biomass (kg FW m^{-2})	10.2 ± 1.3	12.8 ± 1.4	1.31	8.8 ± 0.6	2.6 ± 0.4	7.79**
Damaged buds (%)	24.9 ± 3.4	25.7 ± 3.7	0.15	26.3 ± 3.3	41.0 ± 4.7	2.39**
Surface coverage (%)	93.5 ± 3.1	93.7 ± 2.5	0.05	91.9 ± 2.7	41.1 ± 5.5	7.30**
Water temperature						
Surface	22.3 ± 1.8	24.0 ± 1.9	0.65	22.4 ± 0.6	25.6 ± 0.6	3.17**
1 m	–	–	–	21.2 ± 0.6	24.2 ± 0.6	3.12**
pH	–	–	–	5.9 ± 0.1	6.6 ± 0.1	4.12**
Dissolved oxygen (mg l^{-1})						
Surface	2.7 ± 0.3	1.2 ± 0.2	3.71**	1.8 ± 0.2	2.1 ± 0.3	0.87
1 m	1.0 ± 0.2	0.5 ± 0.1	1.87	0.5 ± 0.1	1.0 ± 0.1	1.97*

Data represent mean ± S.E. ($n = 2$). *$P < 0.05$; **$P < 0.01$.

Fig. 2. Sequence of photographs of the Nelson Pond site before and after release of *C. salviniae* beginning in October, 2001.

3.3. Environmental impacts

Surface water temperatures were the same at control sites and release sites during the pre-release interval ($t = 0.65$; d.f. = 18 and $P = 0.52$) (Table 2). There were no measurements at the 1 m depth during this period. However, during the post-release interval, water temperatures were lower at both depths in control sites ($t = 3.17$; d.f. = 100; $P = 0.002$ and $t = 3.12$; d.f. = 75; $P = 0.002$ for surface and 1 m depth, respectively) (Table 2). We did not record pH during the pre-release interval but this variable was also lower in control sites during the post-release interval ($t = 4.12$; d.f. = 61 and $P = 0.0001$) (Table 2). Dissolved oxygen was greater in control sites on the surface and nearly so at 1 m depth during the pre-release interval ($t = 3.71$, d.f. = 35, $P = 0.0007$ and $t = 1.87$, d.f. = 31, $P = 0.07$, at the surface and 1 m depth, respectively), but was greater in release sites at the 1 m depth during the post-release interval ($t = 1.97$; d.f. = 75 and $P = 0.05$) (Table 2).

4. Discussion

C. salviniae established at multiple sites despite the relatively low number that were released initially. This indicates that rearing and redistribution efforts need not be large scale nor expensive because effective populations can be founded with relatively few adults (Table 1). Insects spread slowly but steadily throughout the sites. Our estimates

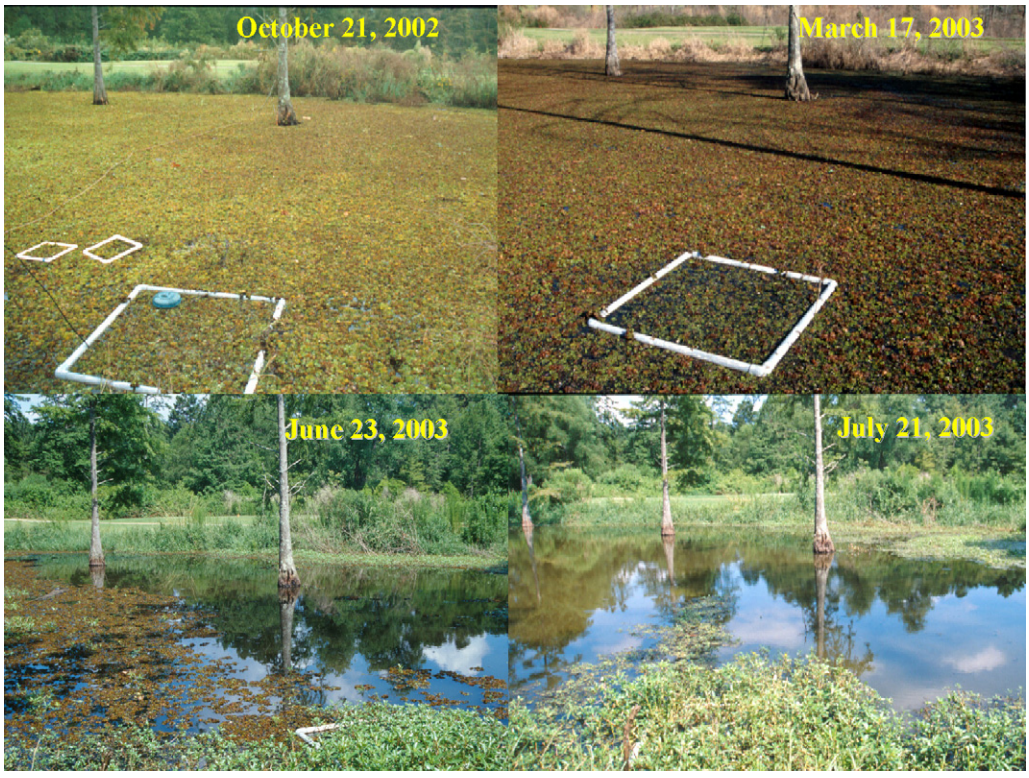

Fig. 3. Sequence of photographs of the Cypress Bend site after release of *C. salviniae* beginning in October, 2001.

were conservative inasmuch as adults likely dispersed beyond our local sample area and were not detected. However, adults rarely fly unless the plants become scarce or suboptimal (Room et al., 1984). Joy et al. (1986) reported a faster rate of dispersal for this species, based on a visual observation of browning plant color. They estimated spread at 25 m after 12 months at some sites; other sites exhibited a spread of 12 m after 7 months. Room and Thomas (1985) reported a rate of movement of 5 m after 6–8 months at sites in Papua New Guinea.

Dramatic reductions of *S. molesta* biomass and surface coverage occurred within 21 months of weevil release (Figs. 2 and 3). Both long term release sites experienced reductions of up to 99% from pre-release levels. At the end of the study, the fresh weight biomass at release sites was 12.5 ± 9.4 and 0 g FW m^{-2} at Nelson Pond and Cypress Bend, respectively. Although only remnant populations of primary and secondary growth forms existed inconspicuously among emergent and floating vegetation around the edges of both release sites, it was common to find *C. salviniae* on these plants. Longer term studies are required to elucidate the equilibrium dynamics between *S. molesta* and *C. salviniae*. Weed infestations did influence water quality in this study although the impacts on the ecosystem from their reductions via biological control are unclear but likely to be positive.

This insect proved host specific in quarantine-based studies prior to release and field surveys found no presence or apparent utilization of native species like *Azolla caroliniana* Will., *Pontederia cordata* L., *Lemna minor* L., *Sagittaria latifolia* Will., and *Ludwigia* sp., or exotic species like *Eichhornia crassipes* (Mart.) Solms and *Pistia stratiotes* L.

As mentioned previously, damage estimates based on bud damage were confounded initially from feeding by *S. multiplicalis* and *S. obliteratis*. However, despite the apparency of the damage, the plants were able to compensate and maintain complete coverage of the water body as evidenced by the lack of change in plant biomass and coverage at control sites. Although *S. multiplicalis* was released and did establish in Australia as part of a biological control project targeting *S. molesta*, its impact was considered to be minimal (Forno, 1987).

C. salviniae has again shown its ability to suppress *S. molesta*, including at a more temperate location that regularly experiences below freezing temperatures during the winter. As in other countries, some maintenance of insect colonies for redistribution to new infestations will probably be needed to satisfy land managers, despite the insect's ability to disperse on their own and find new infestations. The success of this project argues for the deployment of *C. salviniae* to other salvinia infested locations in the southeastern US in order to provide sustainable control of this weed.

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